DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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February 12, 2015

INOVA DIAGNOSTICS, INC.
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SAN DIEGO CA 92131-1638

Re: k141328
Trade/Device Name: Quanta Flash® Ro60
Quanta Flash® Ro60 Calibrators
Quanta Flash® Ro60 Controls
Regulation Number: 21 CFR 866.5100
Regulation Name: Antinuclear antibody immunological test system
Regulatory Class: Class II
Product Code: LLL, JIT, JJX
Dated: January 8, 2015
Received: January 9, 2015

Dear Dr. Lakos:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements

as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Leonthena R. Carrington -A

Leonthena R. Carrington, MS, MBA, MT(ASCP)
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Division of Immunology and Hematology Devices
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Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K141328

Device Name
QUANTA Flash® Ro60
QUANTA Flash® Ro60 Calibrators
QUANTA Flash® Ro60 Controls

Indications for Use *(Describe)*

QUANTA Flash Ro60 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Ro60 autoantibodies in human serum. The presence of anti-Ro60 autoantibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus and Sjögren's Syndrome.

QUANTA Flash Ro60 Calibrators are intended for use with the QUANTA Flash Ro60 Reagents for the determination of IgG anti-Ro60 autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Ro60 Controls are intended for use with the QUANTA Flash Ro60 reagents for quality control in the determination of IgG anti-Ro60 autoantibodies in human serum.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Table of Contents

This summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

Administrative data

Submitter: Inova Diagnostics, Inc
9900 Old Grove Road,
San Diego, CA, 92131

Purpose of submission: New device(s)

Devices in the submission: QUANTA Flash® Ro60
QUANTA Flash® Ro60 Calibrators
QUANTA Flash® Ro60 Controls

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Preparation date: 05/19/2014

Device name (assay kit):

Proprietary name:	QUANTA Flash® Ro60
Common name:	Anti-Ro60 Chemiluminescent Immunoassay
Classification name:	anti-Ro60 antibody, antigen and control

Regulation Description	Antinuclear antibody immunological test system
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	LLL, Extractable Antinuclear Antibody, Antigen And Control

Regulation Number	866.5100	
Device Class	2	
Device name (Calibrators):	Proprietary name:	QUANTA Flash® Ro60 Calibrators
	Common name:	Ro60 Calibrators
	Classification name:	Calibrator, secondary
Regulation Description	Calibrator	
Regulation Medical Specialty	Clinical Chemistry	
Product Code	JIT	
Regulation Number	862.1150	
Device Class	2	
Device name (Controls):	Proprietary name:	QUANTA Flash® Ro60 Controls
	Common name:	Ro60 Controls
	Classification name:	single (specified) analyte controls (assayed and unassayed)
Regulation Description	Quality control material (assayed and unassayed)	
Regulation Medical Specialty	Clinical Chemistry	
Product Code	JJX	
Regulation Number	862.1660	
Device Class	1 (reserved)	

Predicate device: Hycor AUTOSTAT™ II Anti-SS-A Ro ELISA, 510(k) number: K962719

Device description

The QUANTA Flash Ro60 assay is designed to run on the BIO-FLASH® instrument. This platform is a fully automated closed system with continuous load and random access capabilities that automatically processes the samples, runs the assay and reports the results. It includes liquid handling hardware, luminometer and computer with software-user interface. The QUANTA Flash Ro60 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Purified recombinant Ro60 antigen is coated onto paramagnetic beads. The bead suspension is lyophilized and stored in the bead tube. Prior to use in the BIO-FLASH system, the sealed reagent tubes are pierced with the reagent cartridge lid and the beads are rehydrated and resuspended using

resuspension buffer by pipetting up and down with a transfer pipette. The reagent cartridge is then loaded onto the BIO-FLASH instrument. Samples are also loaded onto the instrument in sample racks. Serum samples are prediluted by the BIO-FLASH with system rinse in a small disposable plastic cuvette. Small amounts of the diluted patient serum, the beads, and assay buffer are all combined into a second cuvette, and mixed. This cuvette is then incubated at 37°C. The beads are magnetized and washed several times. Isoluminol conjugated anti-human IgG antibodies are then added to the cuvette, and again incubated at 37°C. The beads are magnetized and washed repeatedly. The isoluminol conjugate is oxidized when Trigger 1 (Fe(III)coproporphyrin in sodium hydroxide solution) and Trigger 2 (urea-hydrogen peroxide in sodium chloride solution) are added to the cuvette, and the flash of light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. The RLU are proportional to the amount of isoluminol conjugate that is bound to the human IgG, which is in turn proportional to the amount of anti-Ro60 antibodies bound to the corresponding beads.

For quantitation, the QUANTA Flash Ro60 assay utilizes a predefined lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. Every new lot number of reagent cartridge must be calibrated before first use, with the QUANTA Flash Ro60 Calibrators. Based on the results obtained with the two Calibrators included in the Calibrator Set (sold separately), an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the instrument signal (RLU) obtained for each sample.

The QUANTA Flash Ro60 kit contains the following materials:

One (1) QUANTA Flash Ro60 Reagent Cartridge
One (1) vial of Resuspension buffer
One (1) Transfer pipette

The QUANTA Flash Ro60 reagent cartridge contains the following reagents for 50 determinations:

a. Ro60 antigen coated paramagnetic beads, lyophilized.
b. Assay buffer – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

The QUANTA Flash Ro60 Calibrators kit contains two vials of Calibrator 1 and two vials of Calibrator 2:

QUANTA Flash Ro60 Calibrators:

- QUANTA Flash Ro60 Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to Ro60 in stabilizers and preservatives.
- QUANTA Flash Ro60 Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL

prediluted, ready to use reagent. Calibrators contain human antibodies to Ro60 in stabilizers and preservatives.

The QUANTA Flash Ro60 Controls kit contains two vials of Negative Control and two vials of Positive Control:

QUANTA Flash Ro60 Controls:

- QUANTA Flash Ro60 Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Ro60 in stabilizers and preservatives.
- QUANTA Flash Ro60 Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Ro60 in stabilizers and preservatives.

Intended use(s)

QUANTA Flash Ro60 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Ro60 autoantibodies in human serum. The presence of anti-Ro60 autoantibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus and Sjögren's Syndrome.

QUANTA Flash Ro60 Calibrators are intended for use with the QUANTA Flash Ro60 Reagents for the determination of IgG anti-Ro60 autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Ro60 Controls are intended for use with the QUANTA Flash Ro60 Reagents for quality control in the determination of IgG anti-Ro60 autoantibodies in human serum.

Substantial equivalence

The QUANTA Flash Ro60 Reagent, the QUANTA Flash Ro60 Calibrators and the QUANTA Flash Ro60 Controls have the same intended use and assay principle as the predicate device.

Comparison to predicate device

QUANTA Flash Ro60 reagent kit

Similarities		
Item	**QUANTA Flash Ro60**	**Predicate Device**
Intended use	Semi-quantitative determination of anti-Ro60 antibodies in human serum	Semi-quantitative detection of anti-Ro60 antibodies in human serum

Similarities		
Item	**QUANTA Flash Ro60**	**Predicate Device**
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Traceability	International Reference Preparation is not available Results are traceable to in-house Standards	International Reference Preparation is not available
Sample type	Serum	Serum
Shelf life	One year	One year

Differences		
Item	**QUANTA Flash Ro60**	**Predicate Device**
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well plate
Antigen	Purified recombinant Ro60 antigen	Native Ro60 antigen, purified from bovine sources
Conjugate	Isoluminol conjugated anti-human IgG	HRP conjugated anti-human IgG
Calibration	Lot specific Master Curve + two calibrators (sold separately)	Single standard (Included in the kit)

QUANTA Flash Ro60 Calibrators

Item	**QUANTA Flash Ro60 Calibrators**	**Predicate Device**
Intended use	QUANTA Flash Ro60 Calibrators are intended for use with the QUANTA Flash Ro60 Reagents for the determination of IgG anti-Ro60 autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.	No separate intended use; calibrator is part of the kit.
Analyte	Anti-Ro60 antibodies	Anti-Ro60 antibodies
Method	QUANTA Flash Ro60 chemiluminescent immunoassay	HYCOR Anti-SS-A/Ro ELISA
Matrix	Human serum, stabilizers, and preservative	Human serum with preservative
Unit	CU (Chemiluminescent units)	U/mL (arbitrary)

Item	QUANTA Flash Ro60 Calibrators	Predicate Device
	(arbitrary)	
Physico-chemical characteristics	Liquid, prediluted, ready to use	Liquid, prediluted, ready to use
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

QUANTA Flash Ro60 Controls

Item	QUANTA Flash Ro60 Controls	Predicate Device
Intended use	QUANTA Flash Ro60 Controls are intended for use with the QUANTA Flash Ro60 reagents for quality control in the determination of IgG anti-Ro60 autoantibodies in human serum.	No separate intended use; controls are part of the kit.
Analyte	Anti-Ro60 antibodies	Anti-Ro60 antibodies
Method	QUANTA Flash Ro60 chemiluminescent immunoassay	HYCOR Anti-SS-A/Ro ELISA
Matrix	Human serum, stabilizers, and preservative	Human serum with preservative
Unit	CU (Chemiluminescent units) (arbitrary)	U/mL (arbitrary)
Physico-chemical characteristics	Liquid, ready to use	Liquid, prediluted, ready to use
Levels	2 (negative and positive)	2 (negative and positive)
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

Analytical performance characteristics

Value assignment and traceability of Calibrators and Controls

There is currently no recognized international standard for the measurement of Ro60 antibodies.
The CDC ANA reference sera #7 (REFERENCE SERUM FOR HUMAN ANTIBODIES TO SSA-60/Ro) and #3 (REFERENCE SERUM, FLUORESCENCE ANTINUCLEAR ANTIBODY, SPECKLED PATTERN) were tested for Ro60 and produced the following results:
CDC ANA #7: >1374.8 CU
CDC ANA #3: 920.0 CU

The QUANTA Flash Ro60 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-Ro60 antibodies with commercial antibody stabilizer, containing preservative. The human serum is obtained from commercial sources and it is tested for markers of infectious substances.
The target CU is achieved through trial dilutions on small scale. Once a dilution is selected, the Calibrators and Control are bulked, tested, and adjusted. Upon completion of the manufacturing process, the Calibrators and Controls are tested on at least two instruments, on at least two lots of reagent cartridge, in replicates of 10 to determine final value assignment.

Calibrator and Control values are directly traceable to in-house Standards that are used to create the Master Curves for the QUANTA Flash Ro60 assay.

List of Ro60 Standards, Calibrators and Controls:

Material	Assigned Value
Ro60 Master Curve Standard 1	4.9 CU
Ro60 Master Curve Standard 2	13.0 CU
Ro60 Master Curve Standard 3	64.1 CU
Ro60 Master Curve Standard 4	145.5 CU
Ro60 Master Curve Standard 5	472.1 CU
Ro60 Master Curve Standard 6	1374.8 CU

Material	Manufacturing Target Value	Manufacturing Target Range
Ro60 Calibrator 1	12 CU	10 – 14 CU
Ro60 Calibrator 2	360 CU	324 – 396 CU
Ro60 Negative Control	10 CU	8 – 12 CU
Ro60 Positive Control	50 CU	40 – 60 CU

Precision

The precision of the QUANTA Flash Ro60 assay was evaluated on 10 samples containing various concentrations of Ro60 antibodies in accordance with CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Procedures - Approved Guideline: samples were run in duplicates, twice a day, for 21 days. Production reagent lots 131007 and 141008 were used for the studies.
Data were analyzed with the Analyse-it for Excel method evaluation software, and within run, between run, between day and total precision were calculated.
Acceptance criteria: Total %CV: < 10%
Results are summarized in the Table below.

QUANTA Flash Ro60			Within Run		Between Runs		Between Days		Total	
Sample ID	Number of replicates	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
110685-1800	84	11.5	0.6	5.0	0.0	0.0	0.5	4.5	0.8	6.8
000674-800	84	16.7	0.9	5.4	0.8	4.8	0.7	4.4	1.4	8.4
110688-1000	84	20.7	1.4	6.9	0.0	0.0	0.9	4.3	1.7	8.1
110689-950	84	25.7	0.9	3.4	0.8	3.3	1.0	4.0	1.6	6.2
110687-1500	84	26.4	0.8	3.1	0.6	2.4	0.5	1.9	1.1	4.4
110687-200	84	142.1	7.7	5.4	4.5	3.1	6.2	4.3	10.8	7.6
110688-85	84	406.3	26.6	6.6	0.0	0.0	21.2	5.2	34.0	8.4
110684-28	84	807.5	25.9	3.2	20.3	2.5	18.6	2.3	37.8	4.7
000674-5.83	84	1181.0	45.3	3.8	49.2	4.2	30.6	2.6	73.6	6.2
110686-55	84	1246.3	67.9	5.4	35.3	2.8	60.8	4.9	97.7	7.8

Reproducibility

Three samples were tested on two different reagent lots, using two different lots of Calibrators, by two operators. Samples were run in quadruplicates, two times a day, for 10 days, to generate 80 data points per sample. Production reagent lots 131007 and 141008, and Calibrator and Control lots 131005 and 131006 were used for the studies. Data were analyzed with the Analyse-it for Excel method evaluation software, and within run, between reagent lots, between calibrator lots, between operators and total precision were calculated.
Acceptance criteria: Total %CV: < 10%
Results are summarized in the Table below.

QUANTA Flash Ro60			Within Run		Between Reagent Lots		Between Calibrator Lots		Between Operators		Total	
Sample	Number of replicates	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	80	11.2	0.2	1.9	0.6	5.8	0.6	5.4	0.5	4.3	0.6	5.3
2	80	21.0	0.5	2.5	1.6	7.4	0.8	3.9	0.7	3.1	1.1	5.4
3	80	100.0	2.7	2.7	5.0	5.0	5.3	5.3	4.2	4.2	5.1	5.1

Limit of Blank (LoB) and Limit of Detection (LoD)

The LoD of the QUANTA Flash Ro60 assay is 519 RLU, which is below the analytical measuring range of the assay. It was determined consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 120 determinations, with 60 measurements on blank samples and 60 measurements of low level samples, per reagent lot.

For determining the LoB, 4 blank samples (System Rinse) from two different lots were run in replicates of five on two reagent lots, once per day, for 3 days. Production reagent lots 131007 and 141008 were used for the studies. Sixty data points were generated on each lot.
The LoB was determined on each lot separately with the *Analyse-it for Excel* software's Reference Interval function, at the 95th percentile, using the non-parametric method, as the dataset showed non-normal distribution (p values < 0.0001). The LoB for lot 131007 was determined as 398 RLU, and for lot 141008 as 452 RLU. The final LoB value is 452 RLU.
For determining the LoD, 4 low level samples (prepared by diluting anti-Ro60 positive samples with System Rinse) were run in replicates of five on two reagent lots, once per day, for 3 days. Production reagent lots 131007 and 141008 were used for the studies. Sixty data points were generated on each lot.
The LoD was determined separately on each lot according to CLSI EP17-A2 guideline. The limit of detection for lot 131007 was determined as 519 RLU, and for lot 141010 as 502 RLU. The final LoD value is 519 RLU.
These values are below the value of the lowest QUANTA Flash Ro60 Master Curve standard, i.e. below the Analytical Measuring Range.

Analytical Measuring Range (AMR)

QUANTA Flash Ro60: 4.9 CU – 1374.8 CU
The AMR is defined by the values of the lowest and highest Master Curve Standards.

Auto-rerun function and reportable results

The BIO-FLASH software has an Auto-rerun option available. If this option is selected, the instrument will automatically rerun any sample that has a result of >1374.8 by further diluting it by 20 fold, thereby bringing the measured value within the AMR. The final result will be calculated by the software by taking into account the additional dilution factor. As the highest value that can be measured is 1374.8 CU, the highest value that can be reported is 27496 CU.

High concentration hook effect

To assess hook effect, measurement signal (relative light units, RLU) was examined for four high positive samples (results above the AMR) before and after automatic or manual dilution. All sera produced significantly higher RLU values (above the AMR) when used "as is" compared to the manually or automatically diluted ones (that were within the AMR), thereby confirming that high positive specimens above the analytical measuring range do not show hook effect up to 19,707 CU in the Ro60 assay (the highest concentration that was tested).

Linearity

The linearity of the AMR was evaluated by a study according to CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Production reagent lot 131007 was used for the study. Three serum samples with various Ro60 antibody concentrations were diluted in 10% increments (from 0% to 90% diluent) to obtain values that cover the AMR. The dilutions were assayed in duplicates. Percent recovery of obtained results was calculated compared to the expected results (based on the dilution factor). Moreover, obtained values were plotted against expected values, and linear regression analysis was performed.
Acceptance criteria:
- Recovery is between 80-120%, or ± 4 CU, whichever is greater.
- For linear regression analysis, slope is between 0.9-1.1, and R^2 is ≥ 0.95.

All three specimens showed dilution linearity individually.

Sample	Test Range (CU)	Slope (95% CI)	R^2
1	148.3 to 1372.2	1.05 (1.02 to 1.08)	1.00
2	15.3 to 178.6	1.00 (0.96 to 1.04)	0.99
3	7.5 to 45.6	1.04 (1.00 to 1.08)	0.99

The combined data yielded the following results with linear regression:

Sample	Test Range (CU)*	Slope (95% CI)	R^2
All	7.5 to 1372.2	1.07 (1.06 to 1.08)	1.00

*The analytical measuring range of the assay is 4.9 CU to 1374.8 CU.

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Three specimens were tested (near-the–cutoff negative: 15.2 CU; weak positive: 40.3 CU; high positive: 161.4 CU). Interfering substances were spiked into every specimen at three different concentrations in 10% of total specimen volume, and the resulting samples were assessed in triplicates with the Ro60 assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents (10% of total). Acceptance criteria for the interference studies were 85% - 115% recovery, or ± 4 CU difference, whichever is greater.

No interference was detected with bilirubin up to 10 mg/dL (recovery: 99% to 109%), hemoglobin up to 200 mg/dL (recovery: 100% to 108%), triglycerides up to 1000 mg/dL (recovery: 89% to 109%),

cholesterol up to 224.3 mg/dL (recovery: 89% to 109%), and RF IgM up to 500 IU/mL (recovery: 90% to 107%).

Cross-reactivity

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, results obtained on 213 of the total 286 control samples that were included in the clinical validation study were assessed. These samples were from patients with autoimmune diseases that are characterized with disease specific autoantibodies, or from patients with positive infectious disease serology. The composition of the cohort and the anti-Ro60 positivity rate is shown in the Table below:

Diagnosis	**Number of samples**	**# pos**	**% pos**
Graves' Disease	10	0	0.0%
Hashimoto Thyroiditis	10	0	0.0%
Celiac Disease	11	0	0.0%
Crohn's Disease	20	1	5.0%
Ulcerative Colitis	20	0	0.0%
HCV	9	0	0.0%
HBV	9	0	0.0%
HIV	5	0	0.0%
Syphilis	10	0	0.0%
Primary Antiphospholipid Syndrome	15	1	6.7%
Vasculitis	1	0	0.0%
Systemic sclerosis	48	1	2.1%
Autoimmune myositis	1	0	0.0%
Rheumatoid arthritis	20	1	5.0%
Autoimmune liver disease*	24	1	4.2%
Total controls	**213**	**5**	**2.3%**
* Samples contain autoimmune liver disease specific antibodies (SLA, F-actin, M2)			

Based on the results, the QUANTA Flash Ro60 assay does not show cross-reactivity with autoantibodies that are present in various autoimmune diseases, or antibodies against infectious agents.

Lot to lot comparison

Twenty-one unique samples and the Positive and Negative Controls (altogether 23 specimens) with various reactivity levels were tested in triplicates with three different reagent lots: 131006, 131007 and 141008. The samples covered the total analytical measuring range of the assay. Results were processed

by linear regression analysis and bias calculation according to CLSI EP09-A2, Method Comparison and Bias Calculation Using Patient Samples; Approved Guideline - Second Edition.
Pair-wise comparisons were performed between lot 131006 vs 131007, lot 131006 vs 14008 and lot 131007 vs 141008, considering individual replicates instead of the mean of replicates.
Acceptance criteria and results are in the Table below. All results were within the acceptance limits.

Acceptance criteria	**131006 vs 131007**	**131006 vs 14008**	**131007 vs 141008**
Weighted r: ≥0.975 for linear regression	0.994	0.996	0.996
Intercept of the regression line (constant bias): ± 15% of cut-off (3 CU)	-1.2	-0.3	1.0
Slope of the regression line (proportional bias): 0.9-1.1	1.0	1.0	1.0
Weighted S y/x: ≤ 0.5	0.095	0.076	0.075
Predicted bias (difference) at cut-off: ±15% (3 CU)	-1.1	0.0	1.3

Sample stability

Six samples, encompassing negative, around the cut-off, and positive samples were tested for up to 21 days at 2-8°C, up to 48 hours at room temperature, moreover, after repeated freeze/thaw cycles up to 3 cycles. One sample had insufficient volume, and could not be tested on days 14 and 21 of the 2-8°C storage study.

Acceptance criteria: 85-115% average recovery.

Claim: up to 48 hours of storage at room temperature, up to 14 days of storage at 2-8^{o}C, and up to 3 freeze/thaw cycles when samples are stored at or below -20^{o}C.

Stability

Shelf life

To establish the initial claim for shelf life, accelerated stability studies were performed for 4 weeks at 37°C ± 3°C, where one week is equal to six months at 5 ± 3°C.
Accelerated stability testing was performed on each of the following sealed components of the QUANTA Flash Ro60 to establish initial stability claim: the beads, the two Calibrators, and the negative and positive Controls. Each week a new sealed component was placed in the incubator, and all components were tested at the end of the experiment together with the one that was stored at 5 ± 3°C. The recovery of the measured values was calculated for each time point (compared to those obtained with 5 ± 3°C stored reagent). All calculations were performed by comparing results of sealed components stored at 5 ± 3°C (control) to those stored at 37 ± 3°C (test) for 1, 2, 3, and 4 weeks, where one week is equal to six months at 5 ± 3°C. Linear regression analysis was performed between recovery values and the number of days.
Acceptance criteria for one year preliminary expiration dating:

- Beads:
With regression analysis, the lower 95% CI interval of the regression line is ≥ 85% at 2 weeks, and no individual data point has ≤ 75% recovery at 2 weeks.
- Controls and Calibrators:
With regression analysis, the lower 95% CI interval of the regression line is ≥ 90% at 2 weeks, and no individual data point has ≤ 80% recovery at 2 weeks.

Beads
Testing was performed on three lots of Ro60 coupled beads using up to 6 characterized samples with various reactivity levels.
All three lots of beads retained > 85% reactivity (considering the 95% CI) after two weeks at 37 ± 3°C, and therefore pass the acceptance criteria for one year expiration date.

Calibrators and Controls
Testing was performed on three lots of Ro60 Calibrators and Controls. All Calibrators and Controls maintained > 90% reactivity (considering the 95% CI) when sored at 37 ± 3°C for 2 weeks, and therefore pass the acceptance criteria for one year expiration dating.

In-use (onboard) stability
Calibrators
Onboard stability claim: 4 calibrations, or 8 hours onboard
During assessing on-board stability, Calibrators were placed uncapped, onboard the instrument, and calibration was performed altogether five times over 8.5 hours. Controls and a panel of characterized patient specimens were run on each calibration curve.
Calibrators are considered stable if all five calibrations performed in the 8.5 hour period are successful, and average Calibrator RLU recovery values are between 90% and 110% compared to the first use.
A total of 5 successful calibrations were performed over an 8.5 hour period. Calibrator RLU values remained within the 90-110% range. Moreover, all Controls and patient panel samples ran within their expected range. This supports the claim that calibrators can be used for up to 4 calibrations over an 8 hour period.

Controls
Onboard stability claim: up to 15 uses, at 10 minutes onboard per use
During assessing on-board stability, 2 vials of each Control were assayed twice a day for a total of 20 runs. The first run (each vial run in duplicate) was used to establish baseline value, and then additional 19 runs (each vial run in singleton) were performed. During runs, the Controls were left uncapped, onboard the instrument for 15 minutes per run. When not in use, the controls were capped, and stored at 5 ± 3°C.
Percent recovery of each value was calculated against the mean of duplicates of each vial from the first run. Controls are considered stable when all values run within their established range, and the linear

regression line obtained by plotting %recovery values against the number of runs stays between 85% and 115% at run 15.
All controls ran within their respective acceptable ranges for all runs. Moreover, the regression line remained between 85% and 115% at run 15 for both Controls. These results support the claim that controls can be used for up to 15 times, at 10 minutes per use.

Reagent Cartridge
To establish the in-use stability of the QUANTA Flash Ro60 reagent cartridge, three lots of cartridges were tested with up to 6 serum specimens (with different reactivity levels) along with the Negative and Positive Controls. The specimens were tested periodically up to 90 days. Percent recoveries were calculated compared to the day zero average values, and linear regression analysis was performed by plotting %recovery against the number of days. The claim was established using the following criteria (using the one that is fulfilled first):
- The stability claim is established at the actual measurement day proceeding the day when the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
- At the actual measurement day preceding the day when 2 data points or ≥2% of the recovery data (whichever is greater) is ≤ 75% or ≥ 125% recovery.

Using these criteria, the in-use (onboard) stability of Ro60 reagent cartridge was set at 49 days.

Real time stability

Real time stability testing was performed at 3, 6, 9 and 12 months on Calibrators and Controls, while testing was performed at 2, 5, 8, and 12 months on the reagent cartridge to support the one year expiration.

For Controls, each control was tested in triplicates at each time point.
- Acceptance criteria: results should fall within their acceptable ranges as it was established at the release of the controls.

Calibrators were tested in triplicates at each time point as it is done during calibration. Averages of the triplicates were compared to the value that was assigned to the Calibrators at release.
- Acceptance criteria: % recovery of the average of the triplicates is between 85 and 115%, and %CV of the triplicates is < 10%

For reagent cartridge, QC panel samples were tested at each time point. This QC panel is used by the QC Department for reagent release and QC.
- Acceptance criteria: results should fall within their respective QC ranges.

All results were within the acceptance limits.

Cut-off, reference range

QUANTA Flash Ro60: Negative <20 CU
Positive ≥20 CU

The reference population for establishing the reference interval for the Ro60 assay consisted of 156 subjects:

Sample Group	N
Apparently healthy blood donors	115
Viral hepatitis positive samples	8
HIV positive samples	5
Syphilis positive samples	5
Rheumatoid arthritis patients	23

All specimens were the same matrix (serum) as specified in the Intended Use. All specimens were unaltered. The cut-off was established in accordance to CLSI C28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. The Analyse-it for Excel software was used to make the calculations. The distribution of the results was non-normal (Saphiro-Wilk $p<0.0001$), so the non-parametric percentile method was used. The 99th percentile of the obtained values calculated as 8193.6 RLU.
Additionally, 12 proficiency testing samples from the College of American Pathologists (CAP) and United Kingdom National External Quality Assessment Service (UKNEQAS) with known consensus/target results were tested to aid in the determination of the cutoff. Taking into account the target results of the proficiency testing samples, the cutoff was increased to 12,000 RLU to ensure optimal differentiation between negatives and positives, and a 20 CU value was assigned to this RLU value. No reference sample tested positive at this cutoff level.

Clinical performance characteristics

Clinical sensitivity, specificity

A cohort of characterized samples, none of which were used for establishing the reference range, was used to validate the clinical performance of the QUANTA Flash Ro60. A total of 475 characterized samples were included in the Validation Set for the QUANTA Flash Ro60. All samples were run on the QUANTA Flash Ro60. The distribution of the cohort and the Ro60 positivity rate is in the Table below:

Patient group	N	Number positive	% positive
Graves' Disease	10	0	0.0%

Patient group	N	Number positive	% positive
Hashimoto Thyroiditis	10	0	0.0%
Celiac Disease	11	0	0.0%
Crohn's Disease	20	1	5.0%
Ulcerative Colitis	20	0	0.0%
HCV	9	0	0.0%
HBV	9	0	0.0%
HIV	5	0	0.0%
Syphilis	10	0	0.0%
Osteoarthritis	20	1	5.0%
Primary Antiphospholipid Syndrome	15	1	6.7%
Secondary Antiphospholipid Syndrome*	15	4	26.7%
Other rheumatic diseases	38	1	2.6%
Vasculitis	1	0	0.0%
Systemic sclerosis	48	1	2.1%
Autoimmune myositis	1	0	0.0%
Rheumatoid arthritis	20	1	5.0%
Autoimmune liver disease**	24	1	4.2%
Total controls	**286**	**11**	**3.8%**
Sjögren's Syndrome	39	26	66.7%
SLE	150	36	24.0%
Total	**475**		
* Patients may have SLE			
** Samples contain autoimmune liver disease specific antibodies (SLA, F-actin, M2)			

The results were analyzed to calculate sensitivity and specificity for SLE (n=150) and Sjögren's syndrome (SS) (n=39) separately, and SLE and SS combined. The secondary APS group was excluded from all calculations.

Clinical sensitivity and specificity of the QUANTA Flash Ro60 in Sjögren's syndrome

n=310		Diagnosis			Analysis (95% confidence)
		SS	Controls (excluding SLE)	Total	
QUANTA Flash Ro60	Positive	26	7	33	Sensitivity = 66.7% (49.8-80.9%)
	Negative	13	264	277	Specificity = 97.4% (94.8-99.0%)
	Total	39	271	310	

Clinical sensitivity and specificity of the QUANTA Flash Ro60 in SLE

n=421		Diagnosis			Analysis (95% confidence)
		SLE	Controls (excluding Sjögren's)	Total	
QUANTA Flash Ro60	Positive	36	7	43	Sensitivity = 24.0% (17.4-31.6%)
	Negative	114	264	378	Specificity = 97.4% (94.8-99.0%)
	Total	150	271	421	

Clinical sensitivity and specificity of the QUANTA Flash Ro60 in SLE+SS

n=460		Diagnosis			Analysis (95% confidence)
		SS or SLE	Controls	Total	
QUANTA Flash Ro60	Positive	62	7	69	Sensitivity = 32.8% (26.2-40.0%)
	Negative	127	264	391	Specificity = 97.4% (94.8-99.0%)
	Total	189	271	460	

Expected values

The expected value in the normal population is "negative". Anti-Ro60 autoantibody levels were analyzed in a cohort of 98 apparently healthy blood donors (52 females and 46 males, ages 17 to 60 years, with an average age of 33.2 years and median age of 29 years) using the QUANTA Flash Ro60. This patient population was different from the one that was used to establish the cutoff, and was only used to assess expected values. With the cut-off of 20 CU, 2 (2 %) of the samples were positive on the QUANTA Flash Ro60. The mean concentration was 6 CU, and the values ranged from <4.9 to 84.8 CU.

Comparison with predicate device

Samples for method comparison analysis included a total of 143 samples from SS and SLE patients and disease controls, with results for 63 samples being within the reportable range of the assay. These samples were tested on both the QUANTA Flash Ro60 and on the predicate ELISA. The predicate ELISA has an equivocal range. The data are presented in two ways; with ELISA equivocal samples as negative in the first table, then as positive in the following table:

Method Comparison, all samples:

Method Comparison (N=143) ELISA equivocal as negative		Ro60 ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash® Ro60 CIA	Negative	93	3	96	Pos. Agree = 93.5% (82.1 – 98.6%)
	Positive	4	43	47	Neg. Agree = 95.9% (89.8 – 98.9%)
	Total	97	46	143	Total Agree = 95.1% (90.2 – 98.0%)

Method Comparison (N=143) ELISA equivocal as positive		Ro60 ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash® Ro60 CIA	Negative	87	9	96	Pos. Agree = 82.7% (69.7 – 91.8%)
	Positive	4	43	47	Neg. Agree = 95.6% (89.1 – 98.8%)
	Total	91	52	143	Total Agree = 90.9% (85.0 – 95.1%)

Method Comparison, samples within reportable range:

Method Comparison (N=63) ELISA equivocal as negative		Ro60 ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash® Ro60 CIA	Negative	16	3	19	Pos. Agree = 93.0% (80.9 – 98.5%)
	Positive	4	40	44	Neg. Agree = 80.0% (56.3 – 94.3%)
	Total	20	43	63	Total Agree = 88.9% (78.4 – 95.4%)

Method Comparison (N=63) ELISA equivocal as positive		Ro60 ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash® Ro60 CIA	Negative	13	6	19	Pos. Agree = 87.0% (73.7 – 95.1%)
	Positive	4	40	44	Neg. Agree = 76.5% (50.1 – 93.2%)
	Total	17	46	63	Total Agree = 84.1% (72.7 – 92.1%)